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EFFECT OF WATER ACTIVITY ON THE MICROBIOLOGICAL STABILITY OF MOBILITY-ENHANCING RATION COMPONENTS

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Garner, NC 27529

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The effect of water activity and pH on the anaerobic growth of Staphylococcus aureus in six mobility-enhancing ration components (MERC), including a beef stick (snack) and five meat sandwiches was determined. These are ready-to-eat rations that can be consumed on the move, eaten without utensils and require no preparation. The MERCs, adjusted to various target water activities and pH, were challenged with a three-strain S. aureus cocktail. Samples were packaged in a clear, permeable, Scotchpack material, overwrapped and sealed in a flexible, high-barrier Meal, Ready-to-Eat pouch containing an oxygen scavenging sachet. Only beef snacks were sealed under 20 mm Hg to simulate commercial practice. All samples were held at 35 degrees centigrade for six months and tested periodically for growth or inhibition of S. aureus, aerobic plate counts, and yeast and molds. S. aureus growth was inhibited in four of the six MERC products tested at a combination of 0.89 water activity and pH 4.8 to 5.4.

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TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

PREFACE

Mobility Enhancing Ration Components (Merc) addressed the need for a ration component system that supports highly mobile troops and are suitable for arctic, jungle, desert, mountain and urban areas under all climatic conditions. Commercial versions of MERC's can be used by campers, hikers, mountain climbers, hunters, fishermen or anyone who needs a convenient, lightweight meal.

These storage stability studies, conducted from March 1998 to May 1999, were supported by a Cooperative Research and Development Agreement (CRADA), between Natick and Goodmark Foods Inc., Garner, NC. The use of a CRADA under the Domestic Technology Transfer Program, which transfers Natick technology to industry, is encouraged to make use of industrial advances and to help strengthen U.S. industries.

The use of trade names in this report does not constitute an official endorsement or approval of the use of any commercial product. This report may not be cited for purpose of advertisement.

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EFFECT OF WATER ACTIVITY ON THE MICROBIOLOGICAL STABILITY OF MOBILITY ENHANCING RATION COMPONENTS

INTRODUCTION

Mobility-enhancing ration components (MERC), such as sandwiches and snacks, are ready-to-eat rations that can be consumed on the move during combat. They can be eaten without utensils and require no preparation. To develop such products, that must be stable for six months at 35°C and three years at room temperature, many factors must be considered, including packaging, microbiological stability and safety, organoleptic quality and long term-storage at ambient temperatures.

Since these products are not sterile, they must be formulated and packaged to prevent growth of pathogenic and spoilage bacteria and molds and still be organoleptically acceptable. To accomplish these goals the rations must be produced at reduced moisture and pH levels and packaged anaerobically. They may also contain antimicrobials such as sorbate. By combining these various factors, particularly water activity (a_w) and pH, it may be possible to inhibit bacterial growth and prevent production of toxin by Staphylococcus aureus, and at the same time improve the ration by increasing moisture levels and decreasing levels of inhibiting agents. High-barrier packaging is essential to maintain reduced oxygen tension provided by oxygen absorbers inside the package.

The challenge organism selected was S. aureus because it is the only bacterial pathogen capable of growth below a_w 0.90 (1, 2, 5, 6). The minimum a_w for its growth in foods is generally considered to be 0.86 (2, 3, 4, 5, 6, 8, 9) and varies depending on the substrate, oxidation reduction potential, temperature, pH, competing microorganisms and chemical preservatives. Because a_w of a food reacts with all these factors, a different preservative system is created in each food system, which may make it possible to elevate the a_w by manipulation of preservatives, and still be inhibitory to S. aureus. For these reasons each MERC ration must be challenged.

The purpose of this study is to provide safety guidelines to manufacturers of MERC products by determining the a_w and pH factors that prevent or influence the growth of S. aureus in the products and thus reduce potential health risks by manipulating those factors.

MATERIALS AND METHODS

Sandwich Production

Beef steaks (Slim Jim^{R}), chicken fajitas, frankfurters, ham slices and nacho cheese meat sticks were produced under proprietary formulas and adjusted for a_w levels and pH by Goodmark Foods Inc., Garner, NC. Tortillas used to make the chicken fajita tortilla wrap were produced for Goodmark Foods at American Institute of Baking, Kansas City, MO. The barbeque chicken filling (Table 1) and the bread were produced and adjusted for a_w and pH at the Soldier Systems Center in Natick. Growth control items for chicken fajitas, frankfurters and ham and cheese were purchased from a local super market. All sandwiches were assembled at Natick to meet end item target a_w levels and pH. The a_w of the meat products was adjusted by a combination of low temperature cooking and air drying for beef steak and nacho cheese stick and by the combination of cooking and glycerol for the barbeque chicken, chicken fajitas, frankfurters and ham slices. Water activity for the shelf stable bread and tortilla products was adjusted by the combination of baking and glycerol.

Table 1. Ingredients of barbeque chicken and sauce.

Barbeque sauce		Barbeque chicken[a]	
Ingredients	%	Ingredients	%
Tomato paste	35.94	Chicken	84.03
Brown sugar	14.90	Chicken broth	5.42
Yellow mustard	11.72	Rice syrup	4.20
Honey	10.42	Glycerol	4.20
Glycerol	7.71	Salt	1.52
Molasses	5.94	Sodium tripolyphosphate	0.42
Ground mustard	3.65	Black pepper	0.21
Vegetable oil	3.13		
Salt	2.03		
Worcestershire sauce	1.98		
Onions, dehydrated	1.54		
Smoke, liquid	0.52		
Garlic powder	0.42		
Red pepper	0,06		
Black pepper	0.06		

[a]Barbeque chicken filling was prepared by adding chicken and barbeque sauce at a ratio of 50:50. Barbeque chicken sandwich was prepared by adding 45g of filling to 70g of bread dough.

The shelf stable bread was produced using the straight dough method. The bread formula for sandwich production is shown in Table 2. The pH of the bread was controlled by the addition of encapsulated glucono-delta-lactone (GDL) to the dough. The dough was mixed for approximately 12 to 15 minutes, until developed. The dough was then set aside for 15 minutes being either enrobed around barbecue chicken filling, or nacho cheese meatsticks using a Rheon KN300 encrusting machine, Huntersville, NC. Dough for frankfurters and

ham and cheese test samples were placed in special bun pans. All products were proofed for one hour at 90°F, at 85% relative humidity in a Hobart convection oven model HGS200 for approximately 20 minutes at 325°F. Tortilla wraps, frankfurters and ham and cheese sandwiches were assembled by hand. Sandwiches were allowed to cool between 80°F to 120°F and packaged in Scotchpak heat-sealable polyester film, 3M Company. Prior to packaging, sandwiches were cut in half to expose a cross section of the interface and surface area between the bread and meat. This facilitated the inoculation of bacteria between the two surface areas.

Table 2. Bread dough formulations.

Ingredients	Not acidified %	Acidified %
Bread flour	50.15	49.93
Water	28.76	28.63
Shortening	8.61	8.57
Glycerol	6.30	6.27
Yeast, instant, dry	2.23	2.23
Salt	1.28	1.27
Sucrose ester	1.00	0.99
Control S (ADM)	0.50	0.50
Gum arabic	0.50	0.50
Glucono delta lactone	0.00	0.45
Calcium sulfate	0.25	0.25
Xanthan gum	0.25	0.25
Potassium sorbate, encap.	0.13	0.13
Cream flavor	0.04	0.03

Media and buffers (10)

Plate count agar (PCA, Difco, Detroit, MI), Baird Parker Agar supplemented with egg yolk tellurite enrichment (BPA, Difco), Potato dextrose agar(PDA) acidified to pH 3.5, and Sterile Buffered Water (SBW, Butterfields phosphate buffer).

Bacterial challenge

Bacteria. The bacteria used were Staphylococcus aureus ATCC 6538, S. aureus, ATCC 8095 and S. aureus, ATCC 27154. All cultures were activated by three daily transfers on PCA at 35°C for 24 hours.

Inoculum cocktail. A three strain S. aureus cocktail was prepared. Cells were washed off PCA, suspended, and diluted in SBW. The suspension of each strain of S. aureus was adjusted turbidimetrically in a Ratio/XR turbidimeter (Hach Co., Loveland, CO.) to 1 x 10^7 colony forming units (CFU)/ml. The cocktail was prepared by mixing together 1 ml of each adjusted suspension. The inoculum was 10 ul of the cocktail.

Challenge. The inoculum (10 ul) was delivered at the interface between the bread and the meat in the case of half sandwiches, and on the surface of the beef sticks (snacks). A Gilson Distriman repeater pipette (Rainin Instrument Co., Inc., Woburn, MA) was used to deliver the inoculum.

Packaging. Samples were packaged in a clear, permeable, Scotchpack material (3M Corporation, St Paul, MN), overwrapped and sealed in a flexible, high-barrier MRE pouch (Cadillac Products, Inc., Sterling Heights, MI) containing an oxygen scavenging sachet (Multiform Desiccants, Inc., Buffalo, NY). Packages were heat-sealed without vacuum with the exception of beef snacks which were heat sealed under 28 mm Hg to simulate commercial practice.

Storage. All samples were held at 35 °C for six months and tested periodically for growth or inhibition of S. aureus, unless growth was evident sooner, at which time the test was terminated.

Sample Preparation for analysis

Samples were aseptically prepared for analysis inside a laminar downflow biological safety cabinet (Nuaire). Before opening, packages of each sample were decontaminated by wiping with gauze pads saturated with 80 % v/v ethyl alcohol. Twenty five to forty gram samples from the site of inoculation were diluted in SBW and stomached for two minutes.

Plate counts

All counts were conducted on duplicate plates using standard microbiological methods (10). Pre-prepared BPA plates were used to recover S. aureus from the cocktail, from inoculated test samples and from uninoculated control samples. Counts were conducted on three samples at each time period. Aerobic plate counts on PCA, and yeast and mold counts on PDA were also conducted on uninoculated control samples. The PCA and BPA plates were incubated at 35°C for 48 h. The PDA plates were incubated at 25°C for 5 days.

Water activity (a_w) and pH measurements

Following the removal of samples for microbiological analysis, the remainder of the sandwich and beef snack were pulverized and homogenized in a dry, sterilized stainless steel blender jar. Water activity (a_w) measurements were made in an AquaLab CX-2 water activity meter (Decagon Devices INC., Pullman, WA 99163). To measure a_w, plastic disposable sample cups (Decagon Devices, Inc.) were half-filled with the pulverized sample and inserted into the instrument. The same samples were used for pH measurements with a Beckman 40 pH meter (Beckman Instruments Inc., Fullerton, CA). 7

RESULTS

The rations were considered microbiologically stable if the initial level of the challenge organisms did not increase more than one log during the six month storage period.

The stability of BBQ chicken at a a_w as high as 0.92 may have been due to the low pH (4.8) as shown in Figure 1. Counts of S. aureus declined immediately and were at nondetectable levels (<100/g) within 7 to 14 days.

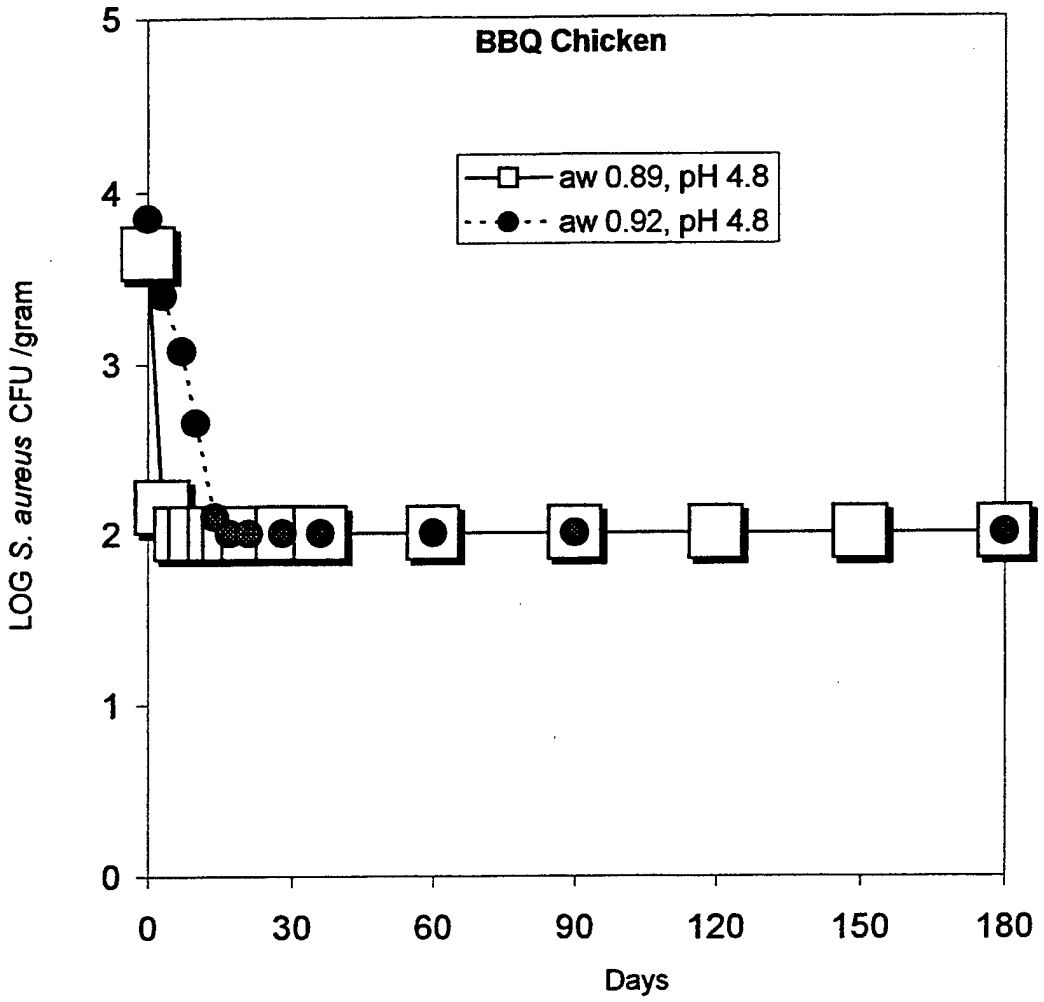

Figure 1. Effect of water activity on growth and survival of a *S.aureus* three strain cocktail in BBQ chicken sandwiches stored at 35 C for six months.

Figure 2 shows that S. aureus will grow on the beef stick (snack) at a_w 0.86 and higher when the pH is as high as six. While growth was rapid and reached spoilage levels at 0.88 a_w and higher, it was delayed and limited at a_w 0.86 and decreased after 14 days to initial levels.

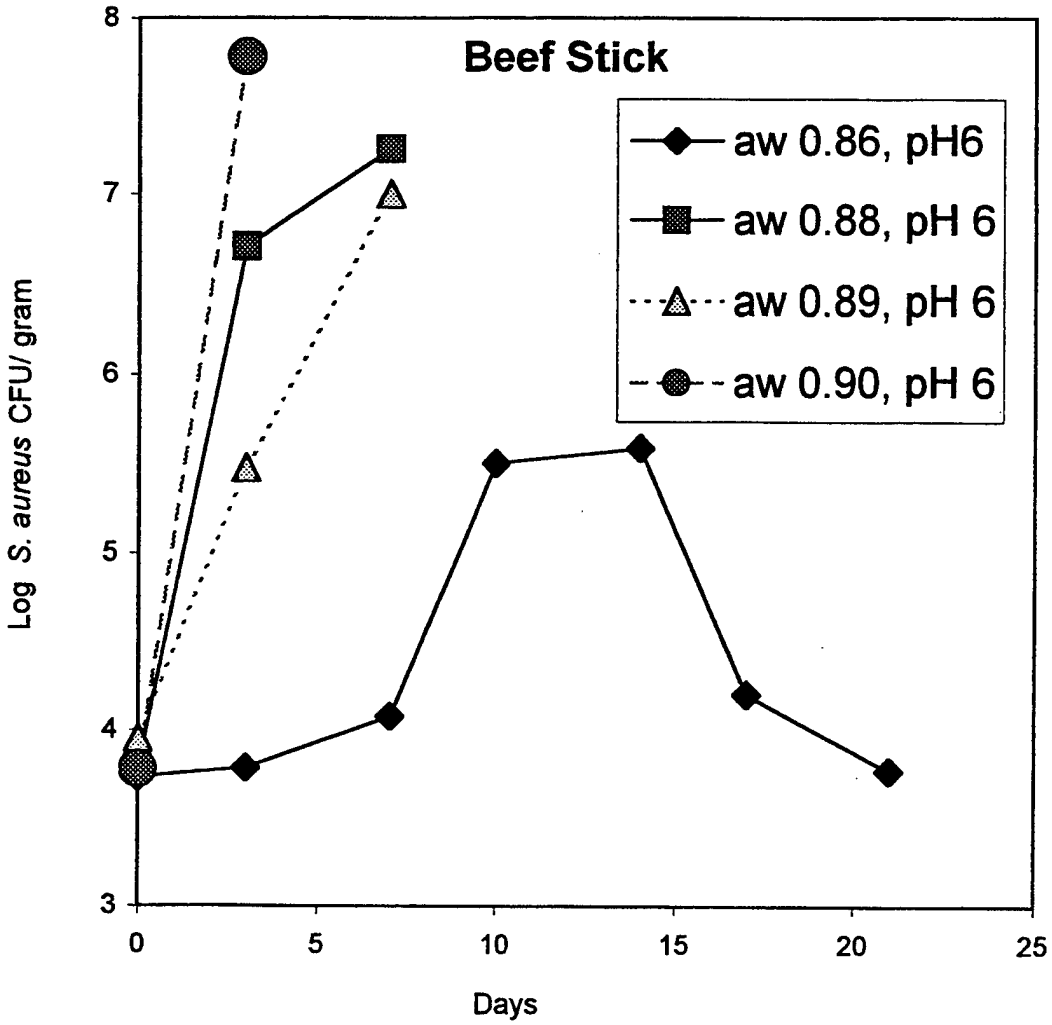

Figure 2. Effect of water activity on growth and survival of an *S. aureus* three strain cocktail on beef sticks stored at 35 C for 21 days

Figure 3 shows that S. aureus grew in the chicken fajitas within 10 days at 0.90 a_w and 0.95 a_w. This was not unexpected at the pH shown.

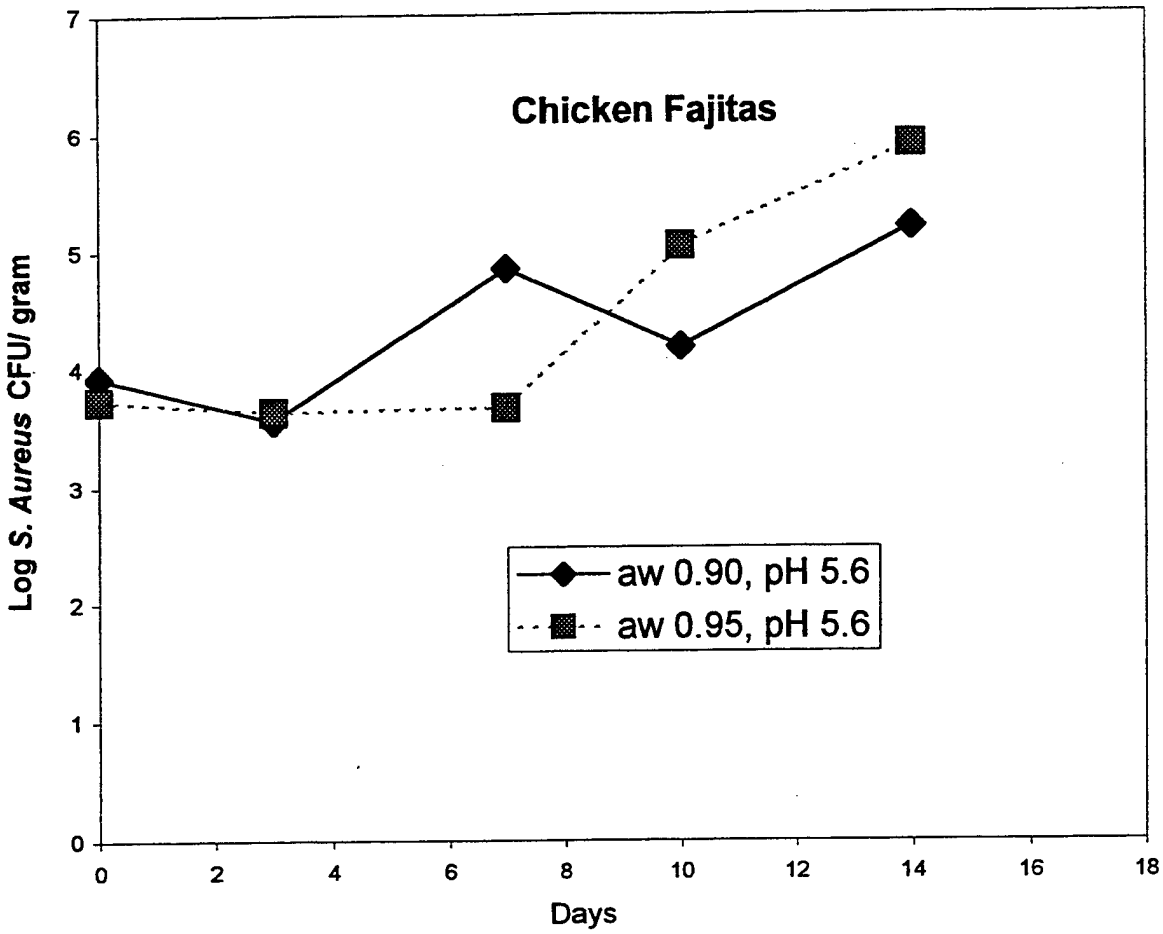

Figure 3. Effect of Water activity on growth and survival of a S. aureus three strain cocktail in chicken fajitas stored at 35 C for 14 days.

The frankfurter and bun remained stable at 35°C for six months at a_w 0.89 as shown in Figure 4. Counts declined to nondetectable levels (<100/g) within 7 days on BPA. Although growth of S. aureus would be expected at 0.89 a_w, it was prevented in the frankfurter and bun, probably by competition and the low pH which was reduced from pH 4.8 to pH 4.5 after 14 days and to pH 4.4 after four months. The competition and reduction in pH was most likely due to the growth of an acid producing Streptococcus spp which was detected on APC's of unchallenged control samples (see Table 3). The Streptococcus spp would not be detected on BPA since it is inhibitory and selective for S. aureus.

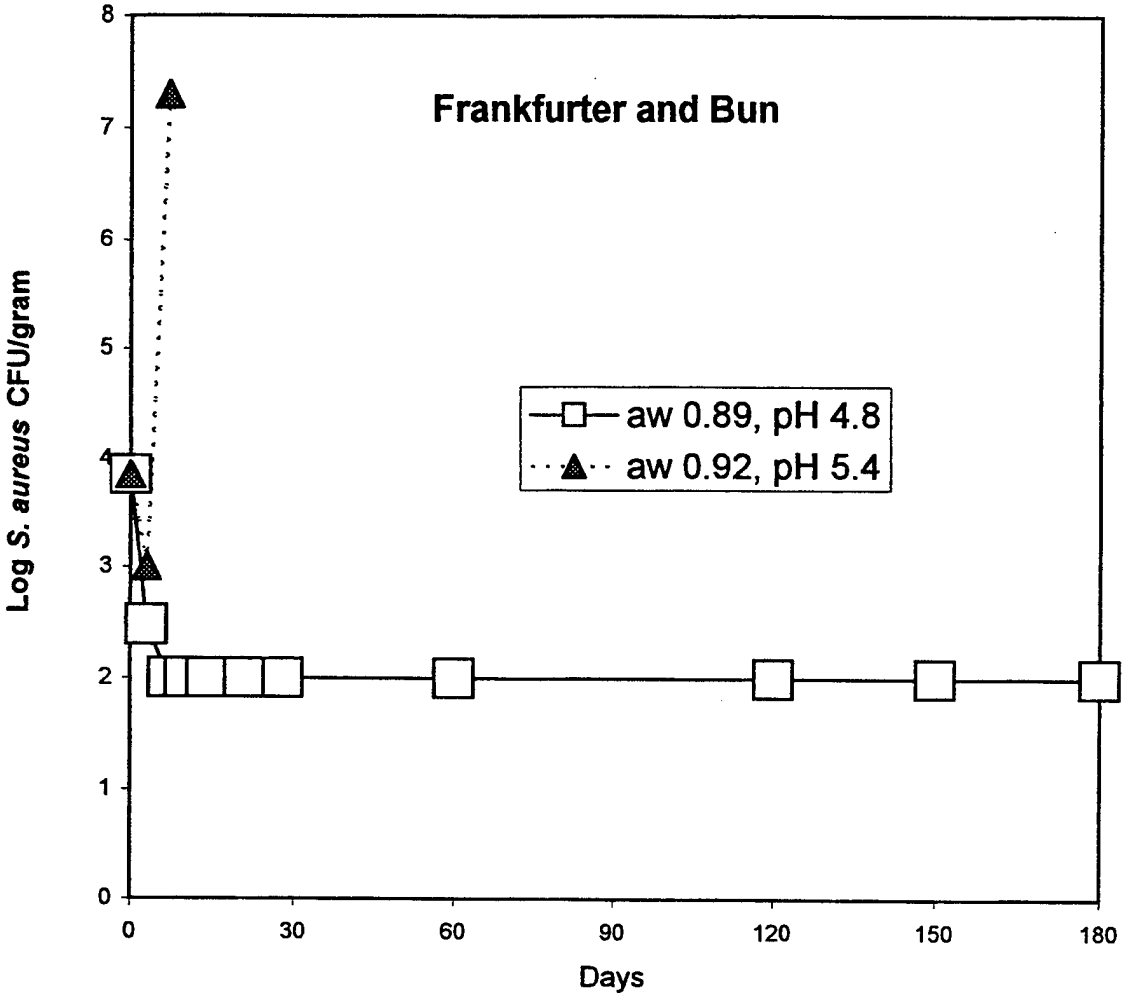

Figure 4. Effect of water activity on growth and survival of a S. aureus three strain cocktail in frankfurters and buns stored at 35 C for six months.

The ham and cheese sandwich was stable at 0.89 ± 0.01 a_w and pH 5.4 ± 0.5 for six months as shown in Figure 5. Counts of S. aureus did not increase but remained stable for 21 days before declining to nondetectable levels (<100/g).

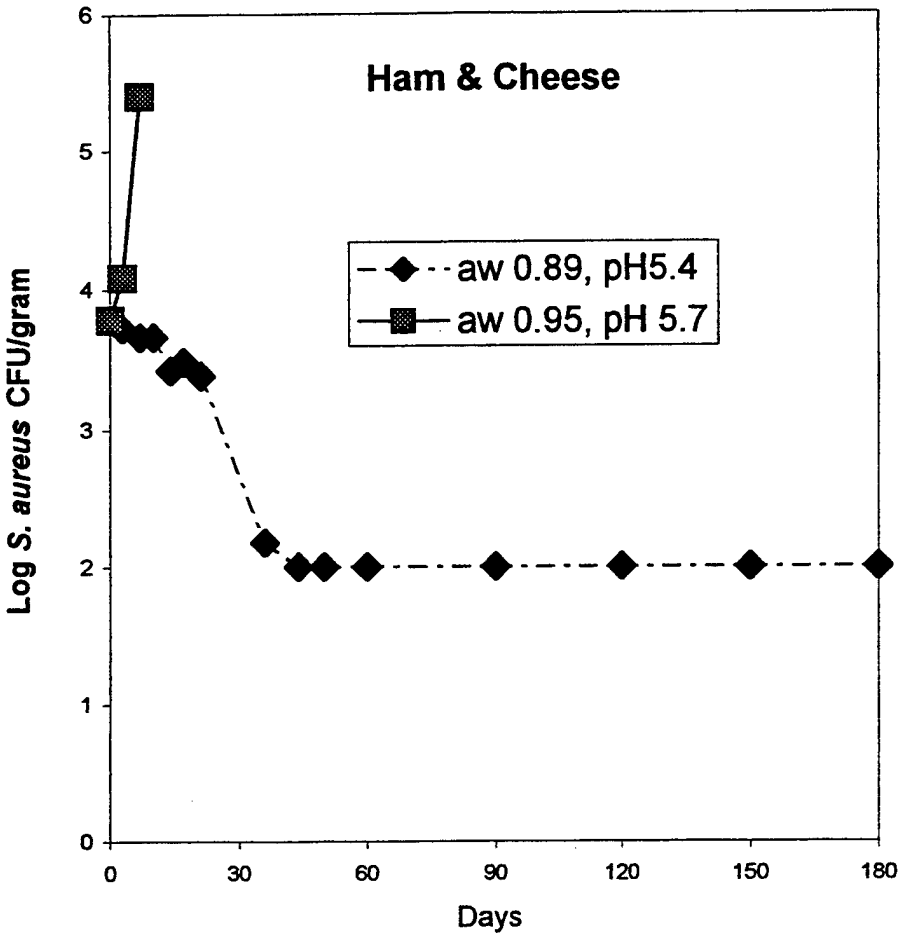

Figure 5. Effect of water activity on growth and survival of a S. Aureus three strain cocktail in ham and cheese sandwiches stored at 35 C for six months.

The nacho cheese and beef sausage in a roll were stable at all three a_w's shown in Figure 6. The combination of a_w and low pH may explain the immediate decline of S. aureus at a_w 0.90. The decline at 0.86 and 0.89 a_w at relatively high pH levels could be due to a synergistic relationship between the a_w and pH or to the presence of organic acids and other inhibitors produced during fermentation of the product.

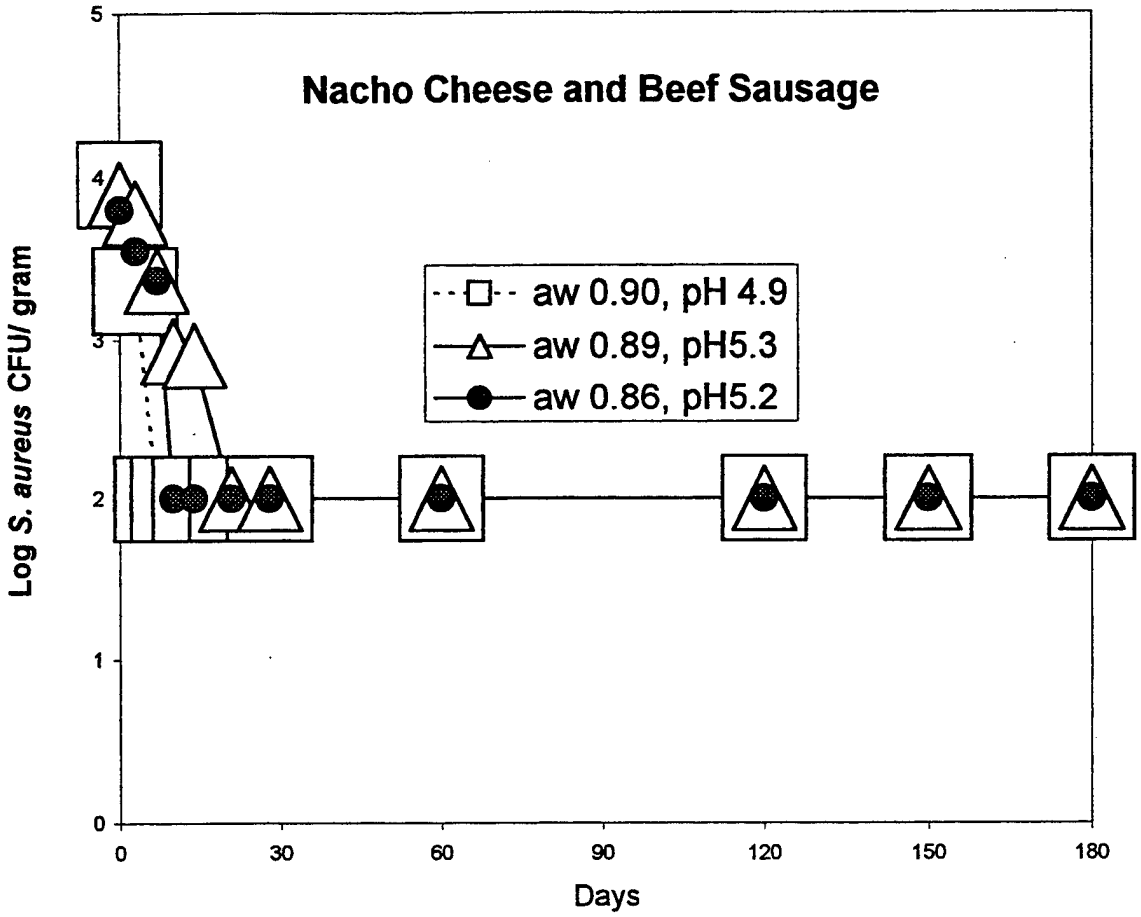

Figure 6. Effect of water activity on growth and survival of a S. aureus three strain cocktail in nacho cheese and beef sausages in rolls stored at 35 C for six months.

Table 3 shows the microbiological quality of uninoculated, unchallenged, control samples of the MERC rations. All samples were randomly selected, packaged and stored at 35°C, exactly under the same conditions as the challenged samples. Initial samples were of excellent quality. Initial counts of yeast and molds (Y/M) and S. aureus (SA) were nondetectable (<10/g). The APC's were also low and detectable only in BBQ chicken sandwiches (0.89 a_w), chicken fajitas, and ham and cheese sandwiches. All counts remained unchanged or declined after 6 months with one exception. The APC's in frankfurters and buns (0.89 a_w) increased to a mean of 7×10^4 CFU/g. This was due to the growth of an indigenous Streptococcus species (gram positive, catalase negative cocci in short chains and pairs). The growth of the Streptococcus species may have been possible because of a moist microenvironment within the package, since it was noted that the bun was soggy and moisture was noted on the surface of the bun at the interface with the package. The moisture level may have been as high as a_w 0.92, since this is the minimum for the growth of Streptococci (1). However, the a_w of the pulverized sample was unchanged. The mean pH was reduced to 4.3, which also indicated the growth of an acid producer (Streptococcus species).

Table 3. Microbiological quality of unchallenged MERC rations.

		Mean[a] CFU/g					
		Initial			Six months		
MERC Ration or sandwich	a_w	APC	Y&M	SA	APC	Y&M	SA
BBQ chicken	0.89	245	195	<10	<10	<10	<10
	0.92	<10	<10	<10	ND[b]	ND	ND
Beef stick (snack)	0.86	<10	<10	<10	--Terminated 21 days--		
	0.88	<10	<10	<10	" 7 days		
	0.89	<10	<10	<10	" 7 days		
	0.90	<10	<10	<10	" 3 days		
Chicken fajitas	0.90	300	<10	<10	--Terminated 10 days--		
	0.95	200	<10	<10	" " "		
Frank and buns	0.89	<10	<10	<10	7.5×10^4	<10	<10
	0.92	<10	<10	<10	--Terminated 7 days--		
Ham & cheese	0.89	250	<10	<10	<10	<10	<10
	0.95	8000	<10	<10	--Terminated 7 days--		
Nacho B&C	0.86	<10	<10	<10	<10	<10	<10
	0.89	<10	<10	<10	<10	<10	<10
	0.90	<10	<10	<10	<10	<10	<10

[a]Average of three samples and two plates per dilution.
[b]Not determined

DISCUSSION

The sandwich formulation was considered resistant to microbial growth and to be microbiologically stable if the initial level of the challenge organisms did not increase more than one log during the six month storage period. However, the test was considered invalid if the average APC and Y&M count in control samples increased more than three log cycles during the duration of the study. As other workers have concluded, a_w is the main factor for determining growth or inhibition because it determines the osmotic stress, and the ability to grow is determined by the degree of that stress (6).

Four of the six MERC products challenged with a three strain S. aureus cocktail were stable at the a_w and pH shown. Two that were not, were the chicken fajitas sandwich which had a $a_w \geq 0.90$ and pH 5.6 and the beef sticks (snacks) at $a_w \geq 0.86$. Although the frankfurters and buns prevented the growth of S. aureus at a_w 0.89 (pH 4.8), the APC in uninoculated controls increased more than 4 log cycles which invalidated the test even though the product may not have been spoiled.

The results obtained with the beef sticks (snacks) validated the Military Specification, MIL-PRF-44394A (11) for this product, which requires a maximum of 0.85 a_w. This a_w value insures the safety of the product by preventing both growth and enterotoxin production by S. aureus. Although S. aureus grew on the vacuum packaged beef snacks at a_w's $\geq$0.86, in this study, which was undesirable and may have caused spoilage, the product was not unsafe because enterotoxin is not produced anaerobically at a_w's <0.90 (1, 2, 5, 16). However, in the event of a package failure, enterotoxins could be produced aerobically at a_w >0.88 (7, 9, 12, 13, 14, 15). The desire to improve the texture of beef sticks (snacks) by increasing the a_w between 0.85 and 0.90, may be possible by concomitantly lowering the pH. Chemical acidulation to as low as pH 5.4 would prevent enterotoxin production under anaerobic conditions (17) even at higher a_w's. However, under aerobic conditions (package failure) pH 4.8 (17) to 5.15 (18, 19) would be required to prevent enterotoxin production.

The migration of moisture and the formation of moist microclimates suitable for growth of pathogens and spoilage microorganisms is a real concern and a threat to safety. This may have occurred in the frankfurter and buns, allowing Streptococci to grow even though the measured a_w of the pulverized samples should have been inhibitory.

Die off after inoculation (see Figures 2, 3 and 6) may be a problem in challenge studies if the inoculum is not high enough. It is often due to shock caused by an abrupt change in environment such as low pH, low a_w, or a combination of the two. While these factors could have contributed to the die-off that occurred in four of the MERC sandwiches, the inoculum level in these studies was high enough to observe either an increase or decrease in levels, even if a 100 fold die-off was observed.

CONCLUSIONS AND RECOMMENDATIONS

More research is required for the formulation of bread and buns and the control of a_w. Particular attention must be given to the migration of glycerine, which is used to adjust a_w, from the bread to the meat and from the meat to the bread. This migration causes sogginess in the bread and changes in a_w which produces an organoleptically unacceptable product. Additional studies may also be warranted in beef snacks at 0.85 a_w, and in chicken fajitas at a_w's lower than 0.90. Challenge studies may also be required at pH levels higher than 4.8 in the BBQ chicken sandwich and frankfurter sandwich if an unacceptable acid flavor is imparted.

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